hERG R56Q channels by i-eag domains could be translated into the environment of cardiac myocytes. hERG R56Q channels were expressed in hiPSC-derived cardiomyocytes (hiPSC-CMs) and electrical properties of the cells were measured with whole-cell patch-clamp. We found that, like in non-myocyte cells, hERG R56Q had fast deactivation kinetics when expressed in hiPSC-CMs. We report here that i-eag domains slowed the deactivation kinetics in hERG R56Q currents in cardiomyocytes by making a direct association with hERG R56Q channels, as measured with FRET spectroscopy, which means that the modulatory role of i-eag domains function in the environment of cardiomyocytes. Isolated eag domains rescued the dysfunction in hERG R56Q channels in the cellular environment of a cardiomyocyte, which shows that they may be useful as a biological therapeutic.

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The Role of the Cyclic Nucleotide Binding Homology Domain in Voltage and Calcium Dependent Gating of EAG Potassium Channels

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Ether-a-go-go (EAG or KCNH1) potassium channels are inhibited by increases of intracellular calcium concentration $[Ca^{2+}]_i$ through a calmodulin (CaM) mediated mechanism. Three CaM binding sites have already been identified on intracellular domains, one close to the N-terminally located eag-domain and two close to the cyclic nucleotide binding homology domain (cNBHD) on the C-terminus. Crystal structures of the cNBHD have revealed that instead of binding cyclic nucleotides the binding pocket is occupied by a short β-strand (intrinsic ligand). In this study we set out to elucidate the role of the cNBHD and intrinsic ligand in CaM regulation of these channels. hEAG1 currents were recorded in Xenopus oocytes using two electrode voltage clamp. Elevation of $[Ca^{2+}]_i$ by application of the Ca^{2+}-ionophore ionomycin (5μM) and SERCA inhibitor thapsigargin (5μM) profoundly inhibited wild-type (WT) hEAG1 currents in a manner that was blocked by the CaM binding peptide mastoparan. Mutation of the intrinsic ligand slowed the time to 80% activation at +40 mV ($t_{80\%,act}$) from 256.35 ± 31.05 ms (n=6) in WT hEAG1 to 1046 ± 15.62 ms (n=3) in Y672A:L674A hEAG1. Substitution of binding pocket residues with large residues also caused similar slowing of $t_{80\%,act}$ (e.g. 682.6 ± 17.9 ms for A609L, n=3). Despite this, none of these mutations caused a reduction in Ca^{2+}-sensitivity. However, deletion of the cNBHD abolished the response to elevated $[Ca^{2+}]_i$ and also had a profound effect on channel gating ($t_{80\%,act}$ 426.6 ± 44.67 ms, n=3). Overall, our results suggest that an intact cNBHD is required for Ca^{2+}-dependent inhibition, but reducing the affinity of the intrinsic ligand for its binding pocket is important for gating not Ca^{2+}-sensitivity.

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Insights into Molecular Basis of hERG Inhibition by Studying a Library of Dofetilide Derivatives

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Understanding the molecular basis of hERG (Kv11.1) inhibition is essential for drug development. Here we systematically analyzed the mechanism and potency of hERG inhibition by a library of 13 dofetilide derivatives1. Currents through hERG channels stably expressed in HEK 293 cells were studied applying whole-cell configuration using planar patch clamp technique2.

The estimated IC50s ranged between 4.3 ± 0.7nM (LUF6200) and 297 ± 77nM (LUF6139). An inverse relation was observed between potency (IC50) and rate of block development. Analysis of the kinetics of block development revealed that potency of dofetilide derivatives is predominantly determined by the drug dissociation rate constant. In general, higher molecular weight led to higher apparent drug affinity (r = 0.9).

LUF6200, the most active compound, has 2 nitro groups in para position of the two aromatic rings and an ethyl moiety at the basic nitrogen atom. Pursuing a Topliss approach at the phenoxy group further supports the positive contribution of the nitro group1, showing that substituents with electron withdrawing groups in para position of the phenyl ring are beneficial for hERG blocking activity. This points towards a pi-pi interaction of at least one of the aromatic rings of dofetilide analogs with respective aromatic amino acids in the channel. Docking of the set of compounds into a homology model of the hERG channel supports this finding. A refined molecular model for dofetilide-hERG interaction based on the novel functional data and mutational studies is proposed.

1. Shagufta et al. Exploring Chemical Substructures Essential for hERG K^+ Channel Blockade by Synthesis and Biological Evaluation of Dofetilide Analogues. ChemMedChem 4, 1722-1732 (2009).
2. Farre, C. & Fertig, N. HTS techniques for patch clamp-based ion channel screening - advances and economy. Expert Opin. Drug Discov. 7, 515-524 (2012).

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Sensitivity of Flecainide Inhibition of hERG Channels to Channel Inactivation

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hERG K^+ channels are sensitive to pharmacological inhibition by many different drugs including Class I and Class III antiarrhythmics. These drugs exhibit differences regarding the reliance of hERG current (IhERG) blockade on channel inactivation: Class Ia (quinidine and disopyramide) and Ic (propafenone) agents are less dependent on inactivation than are Class III methanesulphonanilide compounds (dofetilide and E-4031) (1-3). Flecainide is a Class Ic agent that inhibits IhERG at clinically relevant concentrations (4) and its sensitivity to hERG inactivation has not been systematically investigated. This study was undertaken to evaluate the influence of inactivation on flecainide blockade of hERG, through the use of attenuated-inactivation mutants S631A, N588K and S631A/N588K (3). IhERG was recorded using whole-cell patch-clamp at 37oC from hERG-expressing HEK293 cells. IhERG tails were measured at −40mV after a 2sec depolarizing step from −80mV to +20mV ($n \geq 5$ at each concentration). Flecainide produced a concentration-dependent inhibition of wild-type (WT) IhERG with an IC50 of 1.49μM (CI:1.27-1.74). The IC50 for inhibition of S631A-IhERG [7.49μM (CI:6.33-8.87)] was ~5-fold that for WT-IhERG, whilst that for N588K-IhERG [6.50μM (CI:5.37-7.88)] was ~4.4-fold that of WT-IhERG. The S631A/N588K double mutant, which leads to a greater attenuation of IhERG inactivation than does either individual mutation (3), exhibited an IC50 [19.16μM (CI:15.43-23.80)] ~12.9 fold that of WT-IhERG. Comparison of these data with prior work using these inactivation-mutants (3) is suggestive that flecainide inhibition of IhERG depends on inactivation to a slightly greater extent than Class Ia drugs and the Class Ic drug propafenone, but to a much lesser extent than does E-4031.

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1. Ficker et al. Circ Res 1998; 82:386-95.
2. Perrin et al. Mol Pharmacol 2008; 74:1443-52.
3. McPate et al. BJP 2008; 155:957-966.
4. Paul et al. BJP 2002; 136:717-29.

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High Affinity Block of hERG1 Channels is Weakly Dependent on Inactivation

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Block of hERG1 K^+ channels by many drugs delays cardiac repolarization, prolongs QT interval and is associated with an increased risk of cardiac arrhythmia. A common finding is that high affinity binding of potent hERG channel blockers is inactivation state dependent. The most compelling evidence for this assertion is that point mutations in hERG that inhibit or eliminate inactivation gating (e.g., S620T or G628C/S631C) can reduce drug affinity by >100-fold. Here we examine the link between hERG1 inactivation and blocker affinity in more detail by characterization of concatenated tetramers containing a variable number of subunits harboring mutations that disrupt inactivation. For concatenated tetramers containing only wild-type hERG subunits (wt_4) the IC_{50}'s were 0.20 μM, 0.16 μM, 0.18 μM for cisapride, MK499 and dofetilide, respectively. The presence of a single S620T subunit in a tetramer (ST_1/wt_3) disrupted inactivation gating (+76 mV shift in $V_{0.5}$) similar to a homomeric S620T tetramer (ST_4; +87 mV shift in $V_{0.5}$). For ST_4 channels, IC_{50}'s were 10.6 μM, 6.97 μM, 14.1 μM for cisapride, MK499 and dofetilide, respectively. In contrast, for ST_1/wt_3 channels the IC_{50}'s were 0.60 μM, 1.51 μM, 3.16 μM for the same three drugs. Thus, despite similar effects on inactivation, drug sensitivity of ST_4 channels was significantly less than ST_1/wt_3 channels, indicating that the S620T mutation alters drug binding by a mechanism other than attenuation of inactivation. Unlike S620T, the double mutation G628C/S631C completely eliminates inactivation and concatenated tetramers containing either one or four G628C/S631C subunits induced the same 2.5-fold decrease of cisapride sensitivity (IC_{50}: 0.50 μM and 0.47 μM). Together these findings indicate that preferential binding of drugs to the inactivated state of hERG channels is far less important than is commonly claimed.